Optical characterization of tissue-simulating phantom components at 405 nm

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Abstract—Optical properties of India ink (1950375, Parker, Quink, England) and 1- μ m polystyrene microsphere (07310-15, Polysciences, USA) have been predicted at 405 nm. For this purpose, a single integrating sphere system and spectrophotometric transmission spectroscopy have been used to estimate the radiometric characteristics. Radiometric measurements utilized to retrieve optical coefficients were based on two different methods. The extinction coefficient has been estimated using collimated transmittance. To reconstruct absorption and scattering coefficients, total transmittance, diffuse reflectance and transmittance were measured. Polystyrene albedo was 0.9925 and was 0.1044 for ink. The agreement of PS scattering coefficient in comparison to Mie theory was obvious.

Currently, there is an enormous interest in using a laser in biomedical diagnostics and therapies since it can be considered as a noninvasive method. To evaluate techniques, calibrate equipment, optimize procedures and test theoretical predictions experimentally, optical tissue phantoms are needed [1]. There has been an imperative a medium to calibrate biomedical need for instrumentation such as laser-induced auto-fluorescence using 405 nm laser light mainly used in brain tumor resection, which has given rise to optical phantom construction [2-3]. The optical phantom is a synthetic tissue constructed to simulate the optical properties of present tissues [1]. There are several kinds of material currently used to mimic the absorption property such as blood, food dye and India ink. India ink has been commonly used to construct optical phantom because of its advantages that could be summarized as being inexpensive, spectrally and chemically stable and nontoxic [4-7]. Unfortunately, all prior works aimed to characterize ink optically, revealed the necessity to investigate the optical property of ink due to its interbrand and inter-batch variations [4-7]. Madeson investigated deeply two different brands of ink (Higgins and Regal) and measured the extinction coefficient, anisotropy factor and albedo (ratio between scattering and extinction coefficients) of these two samples at 594nm [4]. The scattering coefficient had been obtained and the albedo of these samples varied between 0.29 and 0.43 besides inter-brand and inter-batch variations [4]. Ninni studied five different ink brands (Pelikan, Higgins,

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Rotring, Staedtler and Koh I Noor) at three wavelengths 632.8, 751 and 833 nm [5]. The inter-brand and interbatch optical characteristics changes were obvious, and the albedo had the largest values at 632.8 nm [5]. On the other hand, the tissue scattering property is a forward scattering and lies between the Mie and Rayleigh scattering. Thus, polystyrene microspheres are considered very appropriate to simulate the scattering feature of tissues [1, 8]. The applicability of their optical prediction based on Mie simulation, is considered the main reason for their widespread use [1, 8]. For more details, most prior research groups deal with this material as a pure scattering material without considering the absorption property [8]. Thus, precise optical investigation of tissuesimulating phantom components plays a vital role in constructing the ideal phantom. On the other hand, optical characterization techniques require both an experimental set-up to retrieve radiometric characteristics and light propagation approaches to extract optical coefficients [9,10]. Accordingly, these methods are categorized as analytical and numerical solutions of the radiative transfer equation (RTE) [10]. The Kubelka-Munk method, an example of analytical solution, is generally implemented with a highly diffusive medium of low absorption like polystyrene and fat emulsions. This method depends on three spectral measurements, collimated transmittance T_c , diffuse transmittance T_d and diffuse reflectance R_d . The scattering and absorption coefficients are expressed as follows [11, 12]:

$$\mu_s = \frac{1}{d} \log(T_d/T_c) + \frac{2.R_d.T_f^{2.log}(T_f^2/T_d)}{d.(T_f^4 - T_d^2)},$$
 (1)

$$\mu_a = \frac{1}{d} \log(T_f^2/T_d) - \frac{2.R_d.T_f^2.\log(T_f^2/T_d)}{d.(T_f^4 - T_d^2)},$$
(2)

where T_f , the Fresnel power transmission coefficient, was introduced to consider the refractive index mismatch. Figure 1 shows the arrangement of diffuse transmittance and reflectance measurement setups that have been in correspondence to the K-M theoretical presumptions. The beam from a fibre-coupled laser (0405L-13A, Integrated Optics, Lithuania) was guided through a convex lens (f =10 cm) and collimated via a 1.5 mm pinhole to the sample of interest. Diffuse reflectance and transmittance were

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measured using a USB spectrophotometer (USB4000 FL, Ocean Optics, U.S.A) [11]. To evaluate collimated transmittance, spectrophotometric transmission spectroscopy was used. That consists of a fibre-coupled laser source (0405L-13A, Integrated Optics, Lithuania), spectrophotometer (USB4000 FL, Ocean Optics, U.S.A), and a cuvette holder (CUV-ATT-DA, Avantes, Netherlands) [11]. Based on the Beer-Lambert law and collimated transmittance, the extinction coefficient of polystyrene samples could be estimated.



Fig. 1. Schematic diagram of an integrating sphere system setup used in PS optical investigations.

The scattering coefficient of PS samples could be calculated via Eq. (1) and diffuse reflectance and transmittance, Table 1.

Table 1. Radiometric characteristics and optical coefficients of a polystyrene microsphere.

C(v/v)	R_d %	T_d %	$T_c\%$	$\mu_t (mm^{-1})$	$\mu_{s}(mm^{-1})$
0.001667	5.6225	8.6050	10.2826	0.227472	0.011761
0.00 200	6.3075	7.5125	6.2931	0.276572	0.052728
0.002333	8.8025	6.0325	3.8515	0.325672	0.097921
0.002667	9.7425	4.9925	2.3571	0.374772	0.137783
0. 00400	14.370	4.0925	0.3307	0.571172	0.350359
Pure Polystyrene	Optical (Coefficients of	147.3	146.2	

Because of using a low volume concentration, the optical coefficients of these diluted samples are linearly related to the volume concentrations for undiluted compounds. Then, a liner relationship between PS coefficients and volume concentration was obvious as can be shown in Fig. 2. Accordingly, the correlations of PS scattering and extinction coefficients with the volume concentration have been found using a curve fitting procedure in Matlab 2014, expressed by:

$$\mu_t = 147.3 * C(v/v) - 0.0284$$
(3)
$$\mu_s = 146.2 * C(v/v) - 0.02403$$
(4)

where μ_t is the extinction coefficient (mm⁻¹), C(v/v) is the volume concentration and μ_s is the scattering coefficient of PS at 405 nm.



Fig. 2. (A) Relation between the PS extinction coefficient and volume concentration, (B) Correlation of the PS scattering coefficient with volume concentration.

Furthermore, the slope of the last figures could be considered as optical coefficients of a pure PS sample without dilution. Then, the pure PS absorption coefficient was 1.102 mm^{-1} , the scattering coefficient was 146.2 mm^{-1} . On the other hand, the PS scattering coefficient, based on the Mie calculation, was obtained and found to be 143.58 mm^{-1} . For more details, the Mie code was implemented with some practical parameters such as the 0.405 µm wavelength of incident light, while the refractive index of a spherical particle was 1.58, the refractive index of water as a solvent was 1.33, the particle diameter and fraction being 1 µm and 0.025, respectively [13]. Finally, the systematic errors turned out to be 1.7%, which confirmed the correspondence of experimental and theoretical findings.

In contrast, numerical solutions such as inverse addingdoubling algorithm (IAD), could be implemented on turbid media such as tissues, ink and polystyrene, avoiding albedo values [14]. To apply IAD, three spectral intensities are required: diffuse reflectance R_d , total transmittance T and collimated transmittance T_c . The RTE is solved repeatedly until experimental radiometric characteristics and numerical solutions are the same?? [14]. For this purpose, a new integrating sphere setup was arranged to enable the application of the IAD algorithm to India ink optical characterization, Fig. 3. The laser beam was focused via a convex lens (f=10 cm) to the ink sample. Total transmittance and diffuse reflectance measurements were applied on the same conditions. The absorption and extinction coefficients could be extracted based on radiometric characteristics, as shown in Table 2. Furthermore, there were some practical and theoretical parameters such as the laser beam diameter of 4 mm, sphere diameter of about 134.6 mm, while the sample and

slides refractive indices were 1.33 and 1.5 respectively and the detector port diameter was about 0.4 mm.



Fig. 3. Schematic drawing of an integrating sphere system setup that used to India ink optical characterization.

Table 2. Radiometric characteristics and optical coefficients of Ink samples.

C (v/v)	R_d %	Τ%	$T_c\%$	$\mu_t (mm^{-1})$	$\mu_a(mm^{-1})$
0.00012	3.101	67.602	64.761	0.04344	0.0319
0.0002	2.778	54.045	51.239	0.06686	0.0519
0.0003	2.602	46.926	43.511	0.08321	0.0659
0. 0006	2.423	33.845	31.532	0.11541	0.0947
0.0009	2.245	20.539	18.675	0.18675	0.1430
Pure Ink	Optical	Coefficients of	148.4	132.9	

Then, the correlation of absorption and extinction with volume concentration were estimated using the fitting curve procedure in Matlab and given by:

$$\mu_t = 148.4 * C(v/v) + 0.03241$$
(5)

$$\mu_a = 132.9 * C(v/v) + 0.02111$$
(6)

Equations (5) and (6) show the relation between both ink extinction and absorption coefficients within the volume concentration. Obviously, the linear relationship could be seen, and slopes of these functions might be considered as pure optical coefficients of black ink, Fig. 4. Therefore, the pure ink extinction coefficient turned out to be 148.4 mm⁻¹, absorption coefficient was 132.9 mm^{-1} , scattering coefficient was 15.5 mm^{-1} and albedo was 0.1044. In the best of our knowledge, Parker ink has not been studied previously at 405 nm [4–7, 15]. Furthermore, the present findings have shown a small scattering of ink in comparison to the absorption coefficient at 405 nm but this value has not been neglected in many cases. Furthermore, the albedo value has been in agreement to prior works that studied other brands of India ink at different wavelengths [4–7].



Fig. 4. (A) Relation between the ink extinction coefficient and volume concentration, (B) Correlation of the ink absorption coefficient and volume concentration.

In conclusion, the present work was an attempt to investigate the optical properties of tissue-simulating phantom ingredients. Black India ink (1950375, Parker, Quink, England) and polystyrene suspension (07310-15, Polybead, Polysciences, USA) have been characterized at 405nm. Parker ink shows a considerable scattering coefficient in comparison to the absorption coefficient, which could not be ignored. Furthermore, black ink could be considered as a turbid medium and only used to simulate a certain tissue which has a similar albedo. On the other hand, the polystyrene absorption property could be neglected in many cases when it is used to mimic a biological tissue which has a valuable absorption coefficient. Finally, precise optical characterization of phantom components would enhance the diagnostic potential of optical instrumentation currently used for discriminating diseased biological tissues.

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